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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 5 FEBRUARY, 1947 No. 2

NUTRITION IN MATERNAL AND CHILD HEALTH PROGRAMS

The nutritional requirements of children and ways of applying the growing fund of knowledge in the field have been matters of fundamental concern to child health workers for well over fifty years. Toward the turn of the century there appeared in the medical literature reports of studies of growth and development of children. In many early inquiries lack of scientific knowledge of nutrition and of the causes of infectious diseases confused the results. Workers in the child health field had to depend to a great extent on empirical clinical judgments and on therapeutic tests.

In 1913, the year after the first State child hygiene division was established, McCollum and Davis, working in Wisconsin, and Osborne and Mendel in Connecticut laid the foundations of the newer knowledge of nutrition. During the next few years methods of laboratory investigation were extended by numerous investigators and soon were applied to the study of the growth and nutrition of children. Not only were some causes of malnutrition and of certain deficiency diseases discovered, but simplified methods of preventing them were demonstrated. The child health program opened the way for the application of both preventive and curative measures in child nutrition.

The successful application of the newer knowledge of nutrition to the feeding of infants and children has been at least partly responsible for the rapid increase in the number of physicians entering the specialty of pediatrics and for the rise of the new professional specialty of the nutritionist. It has led also to instruction in the fundamentals of nutrition in the educational programs of physicians, dentists, nurses, social workers, teachers, and health educators. No book or pamphlet for parents on the care of infants or children today is without its section on nutritional needs as well as on the practical aspects of feeding. The psychologic aspects of feeding children and the effect of emotional factors on the nutrition of infant or child now receive widespread acceptance among pediatricians and other professional workers. Recognition of the influence on both mother and child of nutrition during pregnancy and lactation has come within the past few years. In that short time it has modified the care of maternity patients, especially in the prenatal and postpartal periods. It has also supplied further evidence that the health of mother and child are inseparable.

The practical application of nutrition knowledge takes up a considerable proportion of the time of all workers in the community maternal and child health program. The maternity clinic, the child health conference, the school health service, the pediatric clinic for care of sick children, the home visit of the public health nurse, all offer opportunities for teaching the fundamentals of good nutrition. In the child of school age, nutrition is a part of the school curriculum, with the school lunchroom serving as the laboratory where knowledge is transmitted into action.

In each of these situations the educational process involves a team of workers. The nutritionist member of the team should see that her fellow workers have essential nutrition knowledge and that they are skillful in applying it. Like other specialists, she is on call to deal with unusual problems. The pioneering in community nutrition work was done by a few bold spirits like Frances Stern and Lucy Gillett about thirty years ago. Working together with pediatricians they demonstrated through special nutrition clinics and classes

that healthy growth in children can take place only when children are well fed and in physical condition to convert nutrients into tissues.

Nutrition work in maternal and child health programs extends beyond educational activities. As health agencies have been given responsibility for maintaining standards for hospital care and for administering certain medical care programs for mothers and children, they have appreciated the opportunity thus provided for improving both nutritional status and food habits through serving meals that are appetizing and nourishing. Consequently, nutritionists trained and experienced in dietary management are being employed to act as consultants to maternity homes, convalescent homes for children, and hospitals too small to have the full-time services of a dietitian. Nutrition consultation to educational agencies administering a school lunch program is much in demand with the passage of the National School Lunch Act.

While maternal and child health workers are waiting for education of individuals to "take," they join forces with other public health and nutrition workers in supporting measures that make for mass nutritional protection. Prominent among these measures is the fortification of certain staple foods with appropriate vitamins and minerals as a result of voluntary action on the part of the processor or of legislation.

Public interest in the problems of growth and nutrition of children has moved steadily forward as the newer scientific knowledge has been interpreted to the people themselves and to public agencies that are in a position to influence widespread action in behalf of children. From time to time the Congress has placed administrative responsibilities on the U. S. Children's Bureau for strengthening and extending health services to mothers and children. These grant-in-aid functions have enabled the Federal agency to encourage and assist the States in their efforts to improve the nutritional status and consequently the general health of mothers and children. During the 1920's, several State departments of health used some of the funds available under the Federal Maternity and Infancy Act to employ nutritionists in their Divisions of Child Hygiene. The repeal of this legislation in 1929 and the drastic cut in State appropriations because of the depression of the early 1930's led to the discontinuance of nutrition service by all but three States. Massachusetts, New York, and Connecticut only can point to uninterrupted employment of nutrition consultants for a period of twenty or more years. The maternal and child health part of the Social Security Act of 1935 enabled other State health departments to develop nutrition work as part of their total health services to mothers and children. At latest reports the health departments of forty-five States, the District of Columbia, Hawaii, and Puerto Rico are either employing nutritionists or trying to recruit qualified workers to fill positions already provided for in their budgets. In the closing days of the 1946 session, Congress almost doubled the appropriation for grants to States for maternal and child health services and thereby paved the way for more and better nutrition work in State departments of health. It is anticipated that a substantial proportion of the new money available to States for the current fiscal year will be spent in training personnel for expanded maternal and child health services, including nutrition.

That the stimulation of nutrition work in public health departments has come largely through Federal maternal and child health funds has had both advantages and shortcomings. Probably the major advantage has been that it has placed emphasis on mothers and children. They make up the groups in the population that are in a position to benefit most from improvement in their nutritional status. "Maternal and child health" has been interpreted broadly by the States in outlining the scope of the

activities carried on by nutritionists employed on funds obtained from the Children's Bureau or used for matching those funds. All nutrition services directed primarily toward mothers and children are included whether or not they are carried on as an activity of the maternal and child health division. For example, consultation on the improvement of diets of children suffering from tuberculosis or rheumatic fever is considered entirely appropriate, though the care of those children is the responsibility of another administrative unit or agency of the State. Similarly, in the policies that govern the use of Federal funds for advanced training in nutrition, courses are acceptable that provide advanced study in human nutrition as applied to public health; it is not necessary that the curriculum be confined to the nutrition of mothers and children.

There are two principal limitations in nutrition services financed by maternal and child health funds. Certain appropriate public health nutrition activities, notably the nutrition aspects of industrial hygiene, fall outside the broadest interpretation of maternal and child health. Funds that the Congress has appropriated for services cannot be used for basic research although they can be used in studies that promise to result in an early improvement of services to mothers and children. These limitations to the development of a well rounded State public health nutrition program are less formidable now than they were ten years ago. Increasingly, States are appropriating funds for nutrition work that can be used for purposes not covered by the terms of Federal grants-in-aid. The recent appropriations to the United States Public Health Service for appraisals of the nutritional status of populations and the financial support of research by such private groups as the Nutrition Foundation should mean that services can be based on knowledge of nutrition problems in an area. The State health agencies should soon be in a position to develop well rounded nutrition programs. In these programs, however, services to mothers and children will always be the most important component because nutritional status during the periods of childbearing and growth has such far reaching influence—for better or for worse—on health throughout life.

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PRESENT KNOWLEDGE OF VITAMIN D IN NUTRITION

The group of vitamins D comprises a number of isomeric sterol derivatives which occur in nature chiefly in the animal organism. They can be produced by irradiating corresponding precursors named provitamins D, which are true sterols, with longwave ultraviolet radiation. Their physiologic properties are similar but not quantitatively identical, and their relative effect in different species is not constant. The two most important vitamins D for nutritional purposes are vitamin D_2 (activated ergosterol, calciferol, viosterol) and vitamin D_3 (activated 7-dehydrocholesterol), the form which occurs in nature in the fish liver oils. Provitamins D, such as ergosterol, occur on the other hand in the plant kingdom. The provitamins consist chemically speaking of a substituted cyclo-pentano-perhydro-phenanthrene nucleus. All appear to be similar in having one hydroxyl group, one methyl group, and one higher aliphatic sidechain in the same positions in the molecule, and to differ only in the nature of the aliphatic sidechain, which is branched, may or may not contain an olefinic linkage, and contains 8 or 10 carbon atoms.

The activation of a provitamin D to the vitamin itself involves a rearrangement within the molecule. The central ring of the perhydro-phenanthrene grouping is ruptured and the methyl group, attached to a carbon atom common to two rings in the provitamin remains as a methylene group attached to the surviving ring of the vitamin itself. Activation by ultraviolet radiation is the only method which has been thoroughly investigated, but activation has been reported to be induced also by electron streams, radioactive emanations, x-rays, and high frequency alternating currents. The activation involves a series of reactions rather than a single stage, and if the reaction mixture is irradiated for too long the vitamin D is further transformed into noncalcifying compounds among which are highly toxic materials.

Mammals, including man, are able to synthesize 7-dehydrocholesterol (provitamin D_3) within the body, and presumably this compound can be activated in the skin and then return to the circulation for transport to storage in the liver or to the sites where it performs its activities. In nature the only rich sources of vitamin D appear to be the livers and viscera of fish, and the livers of animals which feed on fish. The milk of mammals contains a little vitamin D, as do milk products such as dried milk and butter, and the yolks of eggs contain definite and rather constant small quantities.

The pure vitamins D are white, odorless, crystals which are soluble in fats and such organic solvents as ether, chloroform, acetone, and alcohol, but are insoluble in water. They exhibit characteristic absorption spectrums with a pronounced band in the region of 265 millimicrons for hexane or ether solutions. They do not possess properties which can be readily utilized as the basis for a satisfactory chemical method of determination and no microbiologic assay has been worked out, so that all assays are carried out with the aid of animals (rats or chicks) and are relatively cumbersome and slow. Although no chemical method of sufficient specificity and sensitivity has yet been developed, some promising steps have been made toward this goal. The relative activities of different vitamins D appear to be the same for different species of mammals, but different for birds. The criterion most commonly used in the assay is the ability of the test substance to promote mineralization of growing bones on a vitamin D- free diet. The bioassays must be carried out with due attention to the species for which the sample is to be used. The rat assay can be used in testing preparations for dosing man and other mammals, but oils to be used on poultry must be assayed with the chick as test animal.

The action of vitamin D is to increase the "net absorption" of the calcium and phosphate of the food, and is exerted in two ways. Directly, the vitamin D increases the permeability of the intestinal mucosa to calcium salts while leaving unchanged the permeability of other membranes. This leads to an increased serum calcium and consequently to a decreased activity of the parathyroid, thereby decreasing the rate of exeretion of phosphate by the kidney. Vitamin D also appears to exert some direct effect on the calcification of growing bone; if large amounts of calcium and phosphate are supplied to the young rachitic animal by injection, then bone of normal density is laid down, but its histologic structure is abnormal. The picture is not clear however in all its details, and especially we need to know more about the qualitative differences between different species in their ability to resist the onset of rickets.

There are few foods which supply vitamin D in the ordinary diet. Butter, eggs, and the liver of mammals supply a little; and the livers of fish are a rich source, but are not common constituents of the ordinary diet. It is not known what amount of vitamin D may be synthesized by man as

a result of exposure to daylight, but it is known that rickets occurs commonly outside the tropical and semitropical zones, and especially in cities where much of the sun's ultraviolet radiation is filtered out by smoke. From this it is concluded that only small quantities are made available to the body in this way. Care must therefore be taken to raise the vitamin D intake of all infants and children. Fairly wide use has been made of milk fortified with vitamin D. Milk is particularly appropriate for fortification with vitamin D since it provides the vitamin together with large quantities of the calcium whose assimilation it aids.

The requirement of vitamin D in childhood has been widely studied, but little is known of the requirement of adults. The National Research Council suggests a daily allowance of 400 I.U. at all ages and this should be sufficient, except in those individuals with abnormalities of mineral metabolism which give rise to "resistant rickets." In this country the occurrence of degrees of rachitic abnormality which can be detected at autopsy by histologic examination of the bones is probably common, but rickets of an intensity producing severe deformity is no longer common, as it was before vitamin D preparations were made available routinely for most children. Rickets of moderate severity is still sufficiently common to demand the active attention of pediatricians in a constant campaign against it (*Nutrition Reviews* **1,** *423 (1943)*).

Care should be used in choosing dosing levels, both for treatment and prevention of rickets, since too large a dose can be toxic, producing an abnormally high calcium level in the serum and overcalcification (*Nutrition Reviews* **3,** *313 (1945)*). In the most severe cases this can cause widespread calcification of soft tissues, and death. A dose many times the recommended daily allowance can be tolerated for long intervals without any toxicity however, and some pediatricians feel that administration of around 250,000 I.U. of vitamin D twice a year is the most satisfactory way to protect infants and children from rickets (*Ibid.* **3,** *209 (1945)*). The serum calcium should be kept under observation in any patient receiving repeated large doses of vitamin D, and if it rises too high the dose should be discontinued. Care should be taken to avoid suggesting inadvertently to an infant or child that fish liver oil or other source of vitamin D is unpleasant. In the absence of such suggestion most children rapidly acquire a liking for these oils, but in the absence of a positive liking for them, there is a possibility of difficulty in administering the dose, and a slight chance of the oil being inspired and causing lipoid pneumonia.

In addition to its use for preventive and curative treatment in man, preparations of vitamin D are important in the feeding of poultry and stock animals. By its routine use as a constituent of the feeds supplied to these animals, egg production has virtually been converted from a seasonal to a year-round basis, and milk production has also been rendered more uniform.

NUTRITION SURVEYS OF PRISONERS OF WAR

To the accumulating information concerning the effects of starvation and impaired diets in those who suffered the privations of war (*Nutrition Reviews* **3,** *225 (1945)*; **4,** *97, 161, 208 (1946)*), C. S. Davidson, H. L. Wilcke, and P. J. Reiner (*J. Lab. Clin. Med.* **31,** *721 (1946)*) add a report on German prisoners observed in a section of upper Austria. One hundred and seventy-one men, representing those who appeared to be the most severely undernourished of a larger group, were chosen for examination. Most of the men had done heavy labor until they were unable to carry on because of lack of

strength. They ranged in age from 16 to 61 years and the average age was 31. The food intake of these men during the two to three months of starvation which they had endured, was calculated with a fair degree of accuracy. The caloric value of the food varied between 650 and 850 calories daily. The diet was deficient chiefly in fat and carbohydrates, the estimated daily content being: protein 40 g., thiamine 0.62 mg., riboflavin 0.64, niacin 8.6, ascorbic acid 10 mg., and vitamin A 6247 I.U.

These prisoners exhibited extreme loss of flesh and generally poor posture. A characteristic lethargy was apparent in all of the men and the facial expressions were dull and rarely changed. Weakness, easy fatigability, vertigo, and syncope were common complaints. Pain in the hips, knees, and ankles was extremely common but bore no relation to the incidence of edema. Diarrhea, probably noninfectious in nature, and paresthesias of the hands and feet, were present in a number of the men. There was a variable amount of pigmentation of the skin which was most marked on the exposed areas of the body. That this was not entirely due to exposure was suggested by its deep color; often it appeared almost black. In addition 32 per cent of the men had follicular hyperkeratosis. Especially in the men with pigmentation, vesicles measuring from 1 to 5 mm. in diameter were observed scattered over the shoulders and upper trunk. These were easily broken and contained clear waterlike fluid. These lesions were assumed to be sweat glands covered with a thin membrane of undesquamated skin. Other skin manifestations sometimes associated with deficiency disease such as purpura, typical pellagrous dermatitis, and nasolabial seborrhea were not seen and evidence of scurvy was not observed.

Bilateral circumcorneal injection together with apparent ingrowth of capillaries into the cornea was observed in 4 men only and this was not accompanied by angular stomatitis or cheilosis. Only 3 showed changes in the color of the tongue. None of these showed papillary atrophy but 8 others had moderate atrophy of the papillae of the tongue.

Moderate hypotension and marked bradycardia were common. The pulse rate in 13 per cent of the men was between 20 and 40 per minute. A phenomenon resembling acrocyanosis was observed in the skin of the hands up to the lower forearm and in the feet to just above the ankle. On a cool morning these areas, particularly the hands, were cold to the touch and presented a mottled cyanotic appearance. During the warmer part of the day the cyanosis sometimes gave way to a mottled reddish flushing but the hands were still unusually cold to the touch. Calf muscle tenderness was observed in three instances. A number of the men had one or more leg ulcers, usually on the lower third of the leg. These were generally clean but showed little evidence of granulation. There were 41 men with varying degrees of dependent edema. In 36 per cent of those examined the serum protein was 5.5 g. per 100 ml. or lower. The presence of edema was not necessarily correlated with the plasma protein level.

From another area comes a report on repatriated American military personnel (G. E. Cartwright and M. M. Wintrobe, *J. Lab. Clin. Med.* **31,** *886 (1946)*). The subjects of this study had been prisoners of the Japanese for periods of from thirty-nine to forty-six months. With two exceptions, all had been imprisoned in both the Philippines and Japan. These men were not studied until four to ten weeks after they had been released, in which interval they had had an opportunity to gain weight and show considerable improvement. Nevertheless, the history they gave was a consistent one and seemed to be reliable. All gave a history of weight loss varying between 5 and 90 pounds. Pellagra as evidenced by cheilosis, glossitis, dermatitis, stomatitis, and diarrhea had been present in some 50 to 70 per cent of the subjects. The incidence of these symp-

toms had been much greater when the men had been in the Philippines and only rarely if at all did this symptom complex develop in Japan while they were receiving soybeans sporadically. An unusual type of change in the skin was a scaly, sometimes erythematous, weeping scrotal dermatitis which was accompanied by extreme tenderness and, in some cases, by edema. About 55 per cent of the individuals with a history of glossitis and stomatitis gave a history of scrotal dermatitis. In only one instance did this symptom appear in the absence of glossitis and stomatitis.

A history suggesting wet beriberi was obtained in 77 per cent of the subjects and a history of dry beriberi was noted in about 50 per cent. These symptoms occurred both in Japan and in the Philippines. Many individuals had both the wet and the dry types but in no individual questioned was a history of both types occurring simultaneously obtained. The dry beriberi was characterized by burning hyperesthesia and paresthesia which were exceedingly severe.

Symptoms suggestive of scurvy, on the other hand, were surprisingly infrequent. Fifteen per cent of the subjects gave a history of bleeding gums but in none of these was the bleeding severe. It is of interest that following intake of a high caloric diet when these men were first liberated, glossitis, stomatitis, and edema reappeared or became more pronounced.

The diets of these former prisoners had consisted, for the most part, of white, brown, or red rice and "green" soup. The rice was issued in amounts varying from 300 to 700 g. per day. Many times whole barley, corn, or soybeans were also mixed with the rice. The "green" soup consisted mainly of water to which small amounts of the tops of green vegetables, grass, or green leaves were added. To many individuals soybeans were unpalatable and even under the severest condition of starvation these were refused because of the nausea, vomiting, and diarrhea which resulted from their ingestion. Meat was rarely obtainable and fish of poor quality was issued only one or two times a month. This dietary regimen led men to resort to eating snakes, rats, cats, dogs, lizards, frogs, grasshoppers, and snails when these were available.

Hemoglobin values below 14 g. per cent were found in 52 per cent of the first 1500 subjects studied. It is of interest that the percentage of men in whom hemoglobin values below the lower limit of normal were observed was greatest during the first week of the survey and gradually decreased, presumably because the longer interval permitted blood regeneration. The blood of 75 subjects was examined in detail. By this time all the men had received vitamin supplements either orally or parenterally. Many had received plasma transfusions and 16 had been given from one to 20 injections of liver extract. The anemia was either mild (from 11 to 14 g. per cent hemoglobin) or moderate in severity (from 8 to 11 g. per cent hemoglobin). Only one instance of severe anemia was observed. In 73 per cent of the 75 men the mean corpuscular volume was greater than normal. In most instances the macrocytosis was mild. In 23 per cent the anemia was normocytic. Only 3 instances of microcytic anemia were found and in 2 of these a definite history of blood loss was obtained. Examination of the blood films was consistent with these observations. Measurement of serum iron in 38 individuals revealed values within the normal range in 45 per cent while in 55 per cent the serum iron was below the normal. In 7 instances, the hypoferremia was extremely severe. There were no instances of hyperferremia. Serum copper determinations revealed values within the normal range in 50 per cent of the cases while in the remainder they were elevated.

Thus it appears that classic signs of nutritional deficiency were much more prevalent in the American soldiers who had been prisoners of the Japanese than in the Europeans examined by Davidson and associates.

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Hemoglobin values below 14 g. per cent were found in 52 per cent of the first 1500 subjects studied. It is of interest that the percentage of men in whom hemoglobin values below the lower limit of normal were observed was greatest during the first week of the survey and gradually decreased, presumably because the longer interval permitted blood regeneration. The blood of 75 subjects was examined in detail. By this time all the men had received vitamin supplements either orally or parenterally. Many had received plasma transfusions and 16 had been given from one to 20 injections of liver extract. The anemia was either mild (from 11 to 14 g. per cent hemoglobin) or moderate in severity (from 8 to 11 g. per cent hemoglobin). Only one instance of severe anemia was observed. In 73 per cent of the 75 men the mean corpuscular volume was greater than normal. In most instances the macrocytosis was mild. In 23 per cent the anemia was normocytic. Only 3 instances of microcytic anemia were found and in 2 of these a definite history of blood loss was obtained. Examination of the blood films was consistent with these observations. Measurement of serum iron in 38 individuals revealed values within the normal range in 45 per cent while in 55 per cent the serum iron was below the normal. In 7 instances, the hypoferremia was extremely severe. There were no instances of hyperferremia. Serum copper determinations revealed values within the normal range in 50 per cent of the cases while in the remainder they were elevated.

Thus it appears that classic signs of nutritional deficiency were much more prevalent in the American soldiers who had been prisoners of the Japanese than in the Europeans examined by Davidson and associates.

This is in keeping with the observations of R. Kark (*Ann. Int. Med.* **25,** *266 (1946)*) on 29 Japanese prisoners of war captured near Burma. Nine of these men had previously been treated for beriberi. The most common lesions seen were cheilosis, angular stomatitis, and invasion of the cornea like that ascribed to riboflavin deficiency. The cheilosis and angular stomatitis were marked and were present in 13 (38 per cent) while glossitis was seen in 4 and edema in 2. The ankle reflex could not be elicited in 3 of the men who stated that they had had beriberi. Biochemical observations showed that the major deficiencies were in hemoglobin levels and in the riboflavin output, the average hemoglobin value being 12 g. per cent, while the urinary excretion of riboflavin was as low as 4 micrograms per hour. The serum protein averaged 5.6 g. per 100 ml., the urinary thiamine 9 micrograms per hour.

VITAMIN LEVELS IN PERNICIOUS ANEMIA

Various vitamin deficiencies have long been known to occur in patients with sprue. D. Cayer, J. M. Ruffin, and W. A. Perlzweig (*Am. J. Med. Sci.* **210,** *200 (1945)*) have reported that multiple deficiencies of the vitamin B-complex are not uncommon in these patients and that vitamin A and carotene levels in the plasma are significantly lower than those encountered in normal individuals. The same authors (*Ibid.* **212,** *179 (1946)*) have extended their investigation to a study of the vitamin levels in pernicious anemia. Eight patients having pernicious anemia were studied, 7 of whom had the characteristic hematologic picture, as well as glossitis, papillary atrophy of the tongue, and early neurologic changes. The eighth patient was in remission. Two of the 7 patients had diarrhea and were passing ten to fifteen stools a day. The oldest patient was 65 years of age, the youngest 37 years. Twenty-five patients with a clinical diagnosis of vitamin B-complex deficiency were studied, together with 30 medical students who were used as normal controls.

The method of study has been discussed in detail in the earlier article. The levels of vitamin A, carotene, and ascorbic acid in the blood of all persons studied were not statistically significant. Two thirds of the patients who had pernicious anemia, as well as 2 having deficiency of the vitamin B-complex, had levels of niacin which were less than the suggested lower limit of normal. The amounts of riboflavin and thiamine excreted before and after test doses were practically the same in the cases of pernicious anemia as in the cases of deficiency of vitamin B-complex; both were significantly lower than those of the normal controls. The values for pyridoxine were the same for all three groups of patients.

The glossitis, cheilosis, slick tongue, and paresthesia of the extremities encountered in patients having sprue or pernicious anemia are frequently indistinguishable from the same observations in patients who have a deficiency of the vitamin B-complex. P. Manson-Bahr (*Lancet* **II,** *317 (1940)*) suggested that deficiency of the vitamin B-complex is common in sprue and pernicious anemia and accounts for these symptoms which are present so frequently.

The work of Cayer and co-workers indicates that the vitamin levels in the blood in the group having pernicious anemia and the group having vitamin B-complex deficiency are closely parallel. However, no apparent correlation was noted between the degree of anemia and the vitamin levels in either group of patients. Recalling the low levels of ascorbic acid and vitamin A in the blood of human beings who do not have any obvious signs of clinical deficiency of these vitamins, one might be hesitant in interpreting these results.

Although the group studied in this series is small, one is impressed by the similarity of the distribution of the vitamin levels in the cases of pernicious anemia and in the cases of vitamin B deficiency. If symptoms of vitamin B-complex deficiency seen in pernicious anemia do not respond to administration of liver extract alone, administration of the vitamin B-complex as an adjunct is justified. This suggestion by the authors has some scientific support.

RELATION OF DIETARY THIAMINE AND PANTOTHENIC ACID CONCENTRATIONS TO THEIR TISSUE LEVELS

Few studies have been made on the relation of the concentration of the B-complex vitamins in the diet to the amounts of these vitamins present in animal tissues. This is of special interest in animals which are widely used for human food. If substantial increases in the vitamin content of meats can be produced by increased dietary concentrations of the respective vitamins, it is possible that the plane of nutrition for these nutrients might be altered somewhat in communities where a particular type of meat is consumed in preference to others by choice or for economic reasons.

The effect of the thiamine content of the diet upon the thiamine content of hog tissues has been studied by W. W. Heinemann, M. E. Ensminger, T. J. Cunha, and E. C. McCulloch (*J. Nutrition* **31,** *107 (1946)*). At weaning, pigs were distributed into five lots with respect to sex, weight, and age. Three lots were maintained on purified diets while the other two were maintained on natural diets. A type of "paired-feeding technic" was employed in which all animals in the purified ration groups received the amount of ration consumed by the animal with the smallest appetite. Likewise the food consumption of the animals in the natural ration groups was limited to that of the animal which consumed the smallest amount of food. Each animal in the three lots which were maintained on purified diets received, respectively, 0 mg., 0.19 mg., or 0.42 mg. of crystalline thiamine hydrochloride per kilogram of body weight per day, while each animal in the two lots maintained on natural diets received, respectively, 0.11 mg. or 0.21 mg. of thiamine per day per kilogram of body weight from a natural source. At the time when the deficient hogs began to lose weight, all the hogs were slaughtered and representative samples preserved for thiamine assay by the thiochrome method. The hogs which received thiamine grew faster and more efficiently per pound of food consumed than those which were thiamine deficient. The hogs which were fed the natural rations were more efficient in utilizing thiamine than those pigs fed the purified rations. Many of the tissues of hogs on the natural diets receiving only 0.11 mg. per kilogram of body weight had a higher thiamine content than those hogs fed purified rations supplemented with 0.19 mg. of crystalline thiamine per kilogram of body weight per day. These thiamine levels were adequate to give normal growth. In hogs fed the higher levels of thiamine daily, the thiamine contents of the respective tissues were higher than on the lower thiamine intakes. In several tissues the thiamine contents were higher for pigs on natural rations receiving 0.21 mg. of thiamine per kilogram of body weight per day than for pigs receiving purified rations plus 0.42 mg. of thiamine per kilogram of body weight per day. The loin and ham were highest in thiamine content followed by shoulder, heart, liver, and kidney in descending order. An interesting observation was made in relation to the riboflavin concentration of the various tissues in corroboration of previous work by other investigators (H. O.

Singher *et al.*, *J. Biol. Chem.* **154,** *69* (*1944* and R. W. Luecke, L. S. Palmer, and C. Kennedy, *Arch. Biochem.* **5,** *395* (*1944*)). The hearts and livers of the pigs fed the thiamine deficient ration contained slightly more riboflavin than those tissues in pigs fed adequate amounts of thiamine.

Somewhat similar studies have been made by P. B. Pearson, V. H. Melass, and R. M. Sherwood (*J. Nutrition* **32,** *187* (*1946*)) on the effect of the pantothenic acid content of the diet of the chicken upon the pantothenic acid content of some of the tissues. The hens used in these experiments were chiefly White Leghorns which had been fed experimental diets differing only in pantothenic acid contents for periods ranging from twelve to twenty-four months. The levels of pantothenate in the diets were 385 micrograms and 1575 micrograms per 100 g. of rations. The latter level may be considered to be adequate to meet the dietary requirements of the chicken. Pantothenic acid assays were made on whole blood, plasma and cells, liver, breast muscle, and leg muscle by a microbiologic method. The amount of pantothenic acid in the whole blood, plasma, and cells was found to be influenced definitely by the pantothenic acid content of the diet. For example the pantothenic acid content of the whole blood from chickens fed the low pantothenic acid diet contained 20.0 micrograms of pantothenic acid per 100 ml., while the blood of the chickens on the high pantothenic acid diet contained 43.6 micrograms per 100 ml. Similar differences were observed for the plasma and cells. In whole chicken blood an average of 86.2 per cent of the pantothenic acid occurs in the plasma compared with 44.3 to 62.6 per cent in mammalia. The differences in pantothenic acid concentrations of the blood in relation to the content of the diet are similar to the observation of R. H. Silber (*J. Nutrition* **27,** *425* (*1944*)) in dogs.

No significant difference was observed in the pantothenic acid content of the livers of the two groups of chickens. Subsequent feeding of a diet containing as high as 3950 micrograms of pantothenic acid per 100 g. did not result in an increase in the pantothenic acid content of the livers. There was a significantly higher level of pantothenic acid in both the breast muscle and the leg muscle of the chickens fed the higher pantothenic acid diet. No further increases were observed when the diet containing 3950 micrograms of pantothenic acid per 100 g. was fed. The addition of pantothenic acid to the diet of chickens which had been receiving a low pantothenic acid intake resulted in an increase in the normal muscle concentration within two to four weeks.

These studies indicate that definite differences in the thiamine and pantothenic acid concentrations in tissues do occur in relation to the levels of these nutrients fed. Under most circumstances the lowest levels of thiamine and pantothenic acid fed by these investigators would not be encountered in commercial feeding due to the high mortality, slow growth, and poor food utilization which would occur. The fact that higher levels of pantothenic acid than are required for adequate nutrition do not increase the pantothenic acid content of muscle tissue indicates that in the chicken higher than normal levels cannot be produced in this manner. On the other hand the results with thiamine indicate that practical gains in the nutritive value of pork may be realized by increasing the thiamine content of the ration. The results of Heinemann *et al.*, although on a somewhat different experimental basis, are in general agreement with the earlier practical feeding studies of R. C. Miller and co-workers (*Nutrition Reviews* **2,** *81* (*1944*)). The latter investigators have shown that the effect of thiamine is evident at ration levels far above the minimum needs.

RENAL CLEARANCE OF AMINO ACIDS

Observations on renal clearance of amino acids are vital to the study of metabolic nitrogen balance. The presence of amino acids in the blood and their virtual absence from the urine indicate that renal retention of these compounds is efficient under ordinary nutritional conditions. Retention of large amounts of amino acids, on the other hand, deserves special attention because of their frequent clinical use. Recent studies have indicated that the glomerular reabsorption of amino acids may vary considerably among compounds when the blood levels are very high.

The experimental technics used in renal clearance studies have been essentially the same among all workers. Dogs have been used throughout as the experimental animals. Solutions of amino acids were injected intravenously or into the femoral arteries of fasting animals. Blood samples were withdrawn at intervals for amino nitrogen analysis, usually by a ninhydrin-carbon dioxide method. Urines were analyzed similarly, but were collected by means of indwelling urethral catheters. Amino nitrogen values were determined after the removal of urea with urease. Glomerular filtration rates were determined by the use of inulin, creatinine, or sodium ferrocyanide, each of which is quantitatively excreted by the kidney.

R. F. Pitts (*Am. J. Physiol.* **140,** *156 (1943);* **140,** *535 (1944)*) studied the renal clearance of glycine, dl-alanine, l(+)-glutamic acid, and l(+)-arginine in dogs. Excretion of alpha-amino nitrogen was followed in relation to the blood levels after the administration of each amino acid. He observed that normally more than 98 per cent of the filtered amino nitrogen was reabsorbed, but that infusions of glycine up to 0.5 g. per minute for twenty minutes resulted in a loss of about half of the amino nitrogen into the urine. The rate of reabsorption rose rapidly at first in proportion to the amount administered, then more gradually as excretion began. The amino nitrogen retention mechanism was thus found to differ from that for glucose, since glucose excretion remains at a very low value until the renal threshold is reached, then increases proportionately with the dose.

The renal clearance of creatine, which was pictured as a glycine derivative, was also studied. Its excretion, which was small normally, was also observed to increase gradually with increasing plasma concentration. When glycine and creatine were present simultaneously, glycine was reabsorbed in relatively greater amounts than creatine. Moreover, glycine administration was observed to increase creatine excretion, whereas the reverse effect was not noted. It was concluded that glycine and creatine were reabsorbed by a common mechanism, glycine preferentially.

dl-Alanine, l(+)-glutamic acid, and l(+)-arginine also competed with creatine, hence were considered to be absorbed by the same mechanism. The exclusion of creatine from reabsorption by the various amino acids was in the following order: glycine, alanine, glutamic acid, and arginine, with arginine exerting only a slight absorption preference. No significance was attached to the fact that the absorptions were inversely related to the chain lengths of the acids.

Similar observations have been made by other workers. E. Goettsch, J. D. Lyttle, W. M. Grim, and P. Dunbar (*Am. J. Physiol.* **140,** *688 (1944)*) observed that high concentrations of glycine and dl-alanine induced excretions of one-fourth to one third of the filtered amino nitrogen. A. G. Eaton, F. P. Ferguson, and F. T. Byer (*Ibid.* **145,** *491 (1946)*) found that injection of 100 mg. of dl-valine per minute for thirty minutes resulted in the loss of about one half of the amino nitrogen, whereas the apparent reabsorption of l(+)-leucine and dl-iso-

leucine was only about 30 to 40 per cent of that filtered.

Pitts, and Eaton and co-workers, have presented an attractive hypothesis of a mechanism of amino acid reabsorption, based upon the combination of the amino acid with some tubular constituent, which later decomposes to release the amino acid to the peritubular interstitial fluid. Interferences in reabsorption, observed when mixtures of amino acids are present, are explained on the basis of competition for inadequate amounts of this tubular constituent, which combines presumably with all in a common manner.

The experimental evidence on which this hypothesis is based is, however, subject to criticism. Although all the authors cited have pointed out that amino nitrogen was being measured, it is logical to assume that the amino acid in question was being excreted. Much of the discussion by Eaton and co-workers was derived from this assumption. That this is probably incorrect is indicated by the work of K. H. Beyer *et al.* (*Am. J. Physiol.* **146,** *330 (1946)*). These authors have studied the renal clearance of *l*(−) tryptophane, *l*(+) leucine, dl-isoleucine, and dl-valine, but have used microbiologic methods for the assay of each amino acid. Their observations are remarkable in that infusions of 30 mg. of tryptophane, 80 mg. of leucine, 170 mg. of isoleucine, or 130 mg. of valine per minute were retained to the extent of 98 per cent or above. Under these conditions it was calculated that the glomeruli were filtering (in gammas): tryptophane 7500, leucine, 16,000, isoleucine 57,000, and valine 55,000 per minute, compared with normal filtration rates of 800, 1600, 1000, and 2300 per minute, respectively.

These results have been confirmed and extended in a separate study by R. H. Silber, A. O. Seeler, and E. E. Howe (*J. Biol. Chem.* **164,** *639 (1946)*). They used a mixture of amino acids ("VujN"), 82 per cent of which consisted of the essential amino acids, 72 per cent in the l form. Intravenous infusion at a similar rate to those employed by K. H. Beyer and co-workers (2 mg. of nitrogen per kilo per minute) resulted in excretion of 4 per cent of the amino nitrogen into the urine. When the rate of administration was raised to the very high level of 12 mg. of nitrogen per kilo per minute for eighteen minutes, 13 per cent was excreted. Phenylalanine, tryptophane, isoleucine, valine, and methionine were retained to the extent of 95 per cent or more according to microbiologic estimate, but larger amounts of the basic amino acids and threonine appeared in the urine; 35 per cent of the lysine was excreted. When 50 per cent of the amino acids were racemic, excretion of amino nitrogen rose to 23 per cent. The amino acid pattern in the urine was different from the pattern of amino acids administered, with the exception of tryptophane. The pattern did not change materially when protein depleted dogs were used, and it was felt that the basic amino acids were being fed in too high concentration, either for proper utilization or for retention by the kidney.

A full explanation of the differences observed among all the groups of workers is not evident at present. There is no doubt that urinary amino nitrogen is increased when amino acids are administered, and that the amount of increase varies considerably among different amino acids. As K. H. Beyer and co-workers point out, excessive plasma concentration of one amino acid may reduce the absorption of related amino acids, so that the excretion of total alpha-amino nitrogen is increased and clearance values may be in excess of that of the particular compound in question. It would be desirable, using the relatively specific microbiologic methods which are now available, to study the effect of each amino acid upon the clearance of several others. This might provide the information necessary to outline the correct mechanisms of renal reabsorption of these compounds.

FERRITIN AND THE ABSORPTION OF IRON

The ability of living systems to pass selectively all sorts of materials across membranes, even at times against steep concentration gradients, is one of the most astounding of biologic phenomena. The nutritionist is deeply concerned with one phase of this phenomenon, the absorption of substances from the food. It must be admitted that although considerable information is available concerning the capacities of the absorptive apparatus, little is known as to the mechanisms of absorption. Inorganic materials are often completely absorbed from the gastrointestinal tract, regardless of bodily needs, and any excess over requirements is excreted in the urine. Calcium and especially iron are prominent exceptions. Relatively little iron is absorbed by the normal adult animal, even after the ingestion of rather large quantities. However, in case of need, as after prolonged blood loss, the absorption of iron may be increased ten- or twentyfold (*Nutrition Reviews* **1,** *154* (*1943*)). It appears to be of importance that little iron be absorbed under normal circumstances since the kidney is unable to excrete more than traces of this metal.

Following a severe hemorrhage the iron absorption rate of the dog does not increase for several days (P. F. Hahn *et al.*, *J. Exp. Med.* **78,** *169* (*1943*)). Apparently some time is required for absorptive mechanism to be set in motion or for resistance to iron absorption ("mucosa block") to be overcome. Hahn *et al.* suggested that the intestinal mucosa may contain a compound capable of combining reversibly with a limited amount of iron, and that this compound in some way acts as a valve, accepting and transferring iron to the body in case of need, but blocking absorption when the iron requirements have been met. They postulated that absorption would be prevented when this compound became saturated with iron, and pointed out that apoferritin might be capable of playing the necessary role. Apoferritin (S. Granick and L. Michaelis, *Science* **95,** *439* (*1942*)) is the iron-free protein component of ferritin (V. Laufberger, *Bull. soc. chim. biol.* **19,** *1575* (*1937*)), an iron-rich compound found chiefly in spleen, but occurring in other tissues as well. Ferritin appears to be one of the major forms in which iron is stored in the body.

S. Granick (*J. Biol. Chem.* **164,** *737* (*1946*)) has demonstrated the presence of ferritin in the intestinal mucosa, and has studied the influence of iron ingestion on the amount of ferritin contained in the tissue. Rapidly growing guinea pigs were utilized so that the demand for iron might prevent undue storage prior to the administration of test doses of iron. The mucosa from 2 to 5 cm. lengths of intestine chosen from different regions of the tract was scraped off and stirred with a 10 per cent cadmium sulfate solution and the sample was allowed to evaporate somewhat overnight. The content of ferritin could be roughly estimated by counting the number of characteristic crystals which cadmium sulfate forms with either ferritin or apoferritin.

In the intestine of untreated guinea pigs, ferritin was just detectable, and, with a few exceptions, was confined to the duodenum. Following the ingestion of 20 mg. of ferrous iron, ferritin appeared in relatively large quantity in the duodenum, and also to a marked but lesser degree in the rest of the small intestine and the pyloric region of the stomach. The cecum and colon, however, contained only minute amounts of ferritin.

The ferritin content of the mucosa rose to a maximum in seven hours after the ingestion of iron and fell slowly over a period of three to six days to the initial low level. No apoferritin could be demonstrated in the mucosa before iron ingestion. Since apoferritin crystallizes as readily as ferritin, Granick concludes that the feeding of iron

leads to the formation of apoferritin as needed.

It seems likely, as a result of these observations, that ferritin is involved in the absorption of iron. Granick believes the observations support the hypothesis of Hahn *et al.* that ferritin is responsible for the regulation of iron absorption. According to this thesis, iron absorption would be accelerated when the ferritin content of the mucosa is low, and delayed when the content is high. It is, however, difficult for this reviewer to understand how the accumulation of an iron complex in the mucosa could obstruct the entry of iron into the blood stream. It is understandable that iron might be accepted by the intestinal mucosa more readily when it is not "saturated" with ferritin, but it is not clear why the transfer of iron from the mucosa to the blood stream would be lessened by such "saturation"; rather the opposite would be expected.

The past few years have seen a rapid accumulation of knowledge of iron absorption and metabolism; perhaps many of the remaining obscurities will soon be clarified.

METABOLIC ALTERATIONS FOLLOWING STARVATION, TRAUMA, AND INFECTION

The metabolic alterations which take place following trauma and during convalescence have been discussed (*Nutrition Reviews* **3,** *86, 253 (1945)*). In one of these the observations of J. E. Howard were described whereby he calculated that without food, at bed rest, and with slight activity such as turning in bed, a vigorous person will lose approximately 10 g. of nitrogen per twenty-four hours per 1.73 square meters of body surface. Allowing 2 g. per day as the margin of error of the determinations, nitrogen losses of more than 12 g. per day may justifiably be considered as due to factors other than simple starvation or rest. A nomograph was constructed on the basis of these calculations to portray the nitrogen balance that might be expected in a healthy individual at bed rest and for comparison with nitrogen losses incited by trauma.

To test the validity of this nomograph J. E. Howard and associates (*Bull. Johns Hopkins Hosp.* **78,** *282 (1946)*) studied 2 healthy young male volunteers who were subjected to bed rest and diets which varied from complete starvation to one and one-half times their calculated requirement. The 2 subjects were under rigorous dietary control and the dietary changes were so arranged that with each alteration all of the constituents under study rose or fell proportionately. Exact twenty-four hour urine collections were made and stools were collected over two and five day periods, the beginning and ending of the periods being marked by the appearance of ingested carmine. Except for a single daily period of fifteen minutes, the subjects remained quietly in bed all of the time. Prior to the study these young men had been in a camp where they were employed at vigorous manual labor.

The first experimental period was one of abrupt starvation followed by gradual feeding and was designed to duplicate as closely as possible the pattern of voluntary dietary intake previously observed in healthy young males after acute injury such as fracture. There was a preliminary control period of ten days, the last five of which were spent in bed, during which diets containing approximately 10 g. of protein nitrogen and 1600 calories per 1.73 square meters of surface area were fed. There followed a two day period of complete starvation; next, two days in which one-fourth the control diet was fed; then, two days with one-half the control diet, and by increases of one fourth of the control

diet in two day periods until one and one-half times the original control diet had been reached. After this a holiday of five days was allowed in which the men were given free choice of food and activity. During this recess most of the lost weight was regained as well as the general sense of fitness and well-being. The second experimental period was begun immediately following this and represented a reversal of the previous one in that the patients were fed during the control period of five days, one and one-half times the diet required for caloric and nitrogen equilibrium at bed rest (15 g. protein nitrogen and 2400 calories per 1.73 square meter surface area). Thereafter, at two day intervals the dietary intake was reduced by a fixed moiety so that in the six periods they received one and a half, one, three-quarters, one-half, one-quarter, and none of the diet.

These observations showed that nitrogen losses both at bed rest and during starvation were slightly greater than would be expected from the hypothetical chart. During periods of decreasing dietary the nitrogen, potassium, and phosphorus were lost in the same proportions as they had been found to exist in muscle protoplasm. During periods of increasing dietary, potassium was held earlier and in greater proportion than was nitrogen. Phosphorus and nitrogen balances, however, continued to reflect the relative proportions in normal muscle. Thus, it would seem that, during starvation, nitrogen and potassium are lost at approximately intracellular proportions but when fed basally and more abundantly, potassium is repleted faster than nitrogen.

Urinary excretion of alpha-amino nitrogen tended to fall when the patients were on submaintenance diets below one-half the calculated requirements. During periods of starvation, there were conspicuous losses of sodium and chloride and the repletion of these substances was slow even when greater than maintenance diets were given. Sharp reductions in concentrations of bicarbonate and chloride in the serum were exhibited as the result of abrupt fasting and slow reduction in the diet. Fasting reduced the capacity of the liver to remove bromsulfalein from the blood.

For the correction of the nitrogen deficit associated with trauma, infection, and burns, attention has been focused on intravenous alimentation and, for this purpose, amino acid mixtures, plasma, and whole blood transfusions have been used. This subject was discussed in an earlier review (*Nutrition Reviews* **4,** *47 (1946)*). Clinically, it is desirable to have available a protein mixture which can be given orally and which, at the same time, is inexpensive. Such a mixture received the attention of H. Y. Spence, E. I. Evans, and J. C. Forbes (*Ann. Surgery* **124,** *131 (1946)*). These investigators used a diet made up of 150 g. of dehydrated beef and liver protein mixture, 150 g. of powdered whole milk, 150 g. of corn oil, 150 g. of sucrose, 150 g. of dextrimaltose, 35 g. of chocolate, and 1000 ml. of water (plus vitamins, especially A, B, C, and D, and iron). This allowed a total intake of at least 3000 calories for each twenty-four hour period. The diet was administered in liquid form, the insoluble materials being held in suspension.

Complete studies in 9 patients are described. In these blood volume was determined as well as total and fractional plasma protein, hematocrit, and hemoglobin. Such observations were carried out at the beginning of each experiment, three or four days thereafter, and again at the end of the experimental period. The nitrogen content of the diet per cubic centimeter was determined and the protein intake for each day calculated from the volume administered. Daily urinary and fecal nitrogen excretion were determined and the nitrogen balance calculated. From the blood volume studies, it was possible to calculate the total circulating plasma, protein, and hemoglobin.

This diet was well tolerated by a patient in whom a partial gastric resection had been made and in another who had been operated

upon because of a perforated peptic ulcer, as well as in one in whom an abdominal gunshot wound necessitated the resection of eight feet of small bowel. Postoperative cases with infection presented the greatest intolerance to the diet. These exhibited a great deal of anorexia, nausea, and vomiting.

In all of the cases, a positive nitrogen balance was achieved. Thus in the patient with partial gastric resection the average nitrogen intake per twenty-four hours was 23.5 g. while the average daily excretion was 19.7 g. In a period of eight days 81 g. of hemoglobin were produced as well as 39 g. of plasma protein and approximately 80 g. of tissue protein. Nitrogen retention averaged, in some subjects, over one third of the nitrogen intake. Where malnourishment and anemia were prominent factors, for every gram of plasma protein formed approximately 2 to 4 g. of hemoglobin and 10 to 30 g. of tissue protein were produced. This corresponds to the observations of F. S. Robscheit-Robbins, L. L. Miller, and G. H. Whipple (*J. Exp. Med.* **77,** *375 (1943)*). In those cases in which the state of nutrition seemed to be good but in whom blood loss had been a prominent factor a greater percentage of the retained nitrogen was used for plasma protein. It is interesting in connection with the pathogenesis of the anemia associated with infection that blood regeneration was impaired in cases where acute infection existed, hemoglobin formation being suppressed even to the extent of hemoglobin loss rather than hemoglobin gain. This occurred despite the nitrogen retention being satisfactory. As an example, one patient was followed for five days before gross wound infection occurred and five days during infection. In the absence of infection, blood regeneration was similar to that manifested by the other uncomplicated cases. During infection hemoglobin and plasma albumin loss occurred whereas the total circulating plasma globulin increased.

W. E. Abbott *et al.* (*Surgery* **20,** *284 (1946)*) report observations on the nitrogen balance in patients suffering thermal burns and describe the effects of diet as well as of testosterone proprionate on the nitrogen equilibrium. They noted that while in some cases it may be possible by means of a very high caloric (60 to 90 calories per kilogram) and high protein intake to prevent or minimize the nitrogen loss, the associated vomiting, diarrhea, and edema which may occur make it inadvisable to force such patients to take large quantities of food at least during the first ten days. More success was achieved with the diet containing one and six-tenths the basal caloric requirement (20 per cent protein). As a rule this level of food intake which was started on the third day after the burn, was well tolerated although, in the severely burned patients, this diet had to be further reduced. During the first few days of therapy the caloric intake was made up entirely of carbohydrate feedings. When such a diet was given the nitrogen loss seemed to be kept at a minimum and the return to a positive nitrogen balance occurred earlier than in those patients receiving a lower caloric and protein intake.

It is well known that the administration of testosterone proprionate is associated with nitrogen retention. The use of this hormone in conjunction with the diet mentioned above seemed to favor nitrogen equilibrium although the investigators admit that this subject requires more rigorous test than they were able to give. Additional carbohydrate in the diet did not seem to produce a significant nitrogen sparing effect. Because P. B. Croft and R. A. Peters (*Lancet* **I,** *266 (1945)*) reported that urinary nitrogen was substantially reduced when dl-methionine supplemented a normal diet following an experimental burn in rats, one patient and several animals were treated by this means, the amino acid being given in an amount equal to 1 per cent of the diet. No confirmation of the observations of Croft and Peters was obtained.

TOXICITY OF THE VITAMINS

Most of the reports in the literature dealing with results of experiments providing varying levels of intake of vitamins have been directed at the prevention or alleviation of deficiency signs and symptoms. The determination of lethal dosage or of toxic manifestations of these substances when large quantities are administered has been the subject of relatively infrequent reports. Few definitive statements are to be found which indicate the relative order of toxicity of the various vitamins or which describe their pharmacologic actions in large doses.

In a study of the toxic effects of niacin, K. Unna (*J. Pharmacol. Exp. Therap.* **65,** *95* (*1939*)) found the LD_{50} of sodium nicotinate for rats and mice to be 5.0 to 7.0 g. per kilogram of body weight when given orally and 4.0 to 5.0 g. per kilogram with subcutaneous administration. The LD_{50} of nicotinamide was about half that of niacin. Death occurred in twelve to thirty-six hours after the administration of lethal doses, with the animals exhibiting generalized paralysis, depressed respiration, and cyanosis. Unna did not note symptoms or pathologic changes of any type in rats, chickens, and dogs during the prolonged administration of as much as 2 g. of sodium nicotinate per kilogram of body weight.

P. Handler and W. J. Dann (*J. Biol. Chem.* **146,** *357* (*1942*)) reported that rats maintained on a diet containing 10 per cent casein had a markedly decreased growth rate when 1 per cent nicotinamide was added to the diet. Inclusion of 2 per cent niacin or 1 per cent trigonelline however had little or no effect on the growth rates of rats on the low casein diet. Inhibition of growth by nicotinamide was prevented by the administration of methionine or choline plus homocystine but not by choline, betaine, homocystine, or cystine alone. The authors cited evidence to indicate that nicotinamide was the immediate precursor of trigonelline and that methionine served as the source of methyl groups for the conversion of nicotinamide to trigonelline. It was concluded that growth inhibition brought about by the administration of large quantities of nicotinamide was the result of deprivation of the animal's supply of methyl groups for trigonelline synthesis.

F. G. Brazda and R. A. Coulson (*Proc. Soc. Exp. Biol. Med.* **62,** *19* (*1946*)) have compared the toxic effects of niacin and related compounds given by subcutaneous injection to young rats. The LD_{50} of niacin was 5.0 g. per kilogram of body weight. The relative toxicity of related compounds indicated that nicotinamide was three times, pyridine five times, and coramine (N'N-diethylnicotinamide) twenty-one times as toxic as niacin. Methylation decreased the toxicity of coramine and nicotinamide, increased that for pyridine, and had little effect on the toxicity of niacin. Indications were that the toxicity of the nonmethylated compounds is related to their molecular configurations rather than to depletion of body stores of methyl donors in the detoxification process.

The toxicity of niacin revealed by these studies may be compared with that of other water soluble vitamins as recorded in other papers in the literature. Thiamine is apparently somewhat more toxic than niacin. Two hundred and fifty mg. per kilogram of body weight given intravenously produces death in rats (H. Molitor and W. L. Sampson, *Merck's Jahresberichte* **50,** *51* (*1936*)). Pyridoxine is of about the same degree of toxicity as niacin, the lethal dose in rats being 3.0 g. per kilogram of body weight (K. Unna and W. Antopol, *Proc. Soc. Exp. Biol. Med.* **43,** *116* (*1940*)). There is no information in the literature to indicate that riboflavin, pantothenic acid, para-aminobenzoic acid, or ascorbic acid produce toxic effects of any type even when administered in large doses to experimental animals. However, ascorbic acid in large quantities (1 g. per

day) produces diuresis in human beings, an effect which is not due to acidification (M. A. Abbasy, *Biochem. J.* **31,** *339* (*1937*)).

Toxic effects of the fat soluble vitamins, A and D, have been demonstrated repeatedly and are manifest with the administration of smaller doses than is necessary to produce signs of toxicity with the water soluble vitamins. Crystalline vitamin A acetate produces retarded growth, rarefaction of bones, exopthalmos, and subcutaneous and intramuscular hemorrhages in young rats given doses exceeding 530,000 I.U. per kilogram of body weight per day (T. Moore and Y. L. Wang, *Biochem. J.* **39,** *222* (*1945*)). Vitamin D in doses greater than 20,000 I.U. per kilogram of body weight per day has been demonstrated to cause signs of toxicity in experimental animals and in man. Evidences of toxicity include loss of weight, diarrhea, vomiting, signs of renal damage, increased calcium content of serum, and the appearance of calcified areas in the viscera (C. A. Ashford, *Ibid.* **24,** *661* (*1930*)). Although the naturally occurring vitamins K have been shown to be nontoxic even in excessive doses, the synthetic preparations, 2-methyl-1,4-naphthoquinone and 2-methyl-1,4-napthohydroquinone, produce evidences of toxicity when administered in large doses to dogs, rabbits, and human beings (H. Molitor and H. J. Robinson, *Proc. Soc. Exp. Biol. Med.* **43,** *125* (*1940*)). In dogs the toxic dose of these substances varies from 30 to 60 mg. per kilogram of body weight.

The general statement may be made that the vitamins are relatively nontoxic. For those which have been shown to produce death or evidences of toxicity, the ratio of the quantity required daily to maintain optimum nutrition to the lethal or toxic dose varies from 1:600 for 2-methyl-1,4-napthoquinone to 1:60,000 for pyridoxine. This ratio is 1:2000 for vitamin D, 1:5000 for niacin, 1:7500 for vitamin A, and 1:25,000 for thiamine. Sufficient latitude in dosage seems to exist for each of the vitamins to preclude all effects from administration to man for nutritional purposes. Yet these substances are readily available and are often administered in quantities far exceeding daily requirements. Evidences of hypervitaminosis A, D, and K have been observed in man and are recorded in the literature. It is important therefore to keep in mind that toxic doses can and have been given.

Certain of the vitamins may possess pharmacologic actions which are not apparent when administered in the small quantities usually provided in nutritional experiments. This is an additional reason why further careful studies of the effects of large dosage of these substances would be of value. Furthermore, such knowledge might give leads to modes of action or suggest other uses of the vitamins in the treatment of disease.

FAILURE OF VITAMIN K IN TREATMENT OF HYPERTENSION IN DOGS

Kidney extracts and marine oils have therapeutic value in experimental hypertension in dogs and rats and have been used with promising results in cases of essential hypertension in man (*Nutrition Reviews* **1,** *20* (*1942*); **1,** *141* (*1943*); **3,** *331* (*1945*)). Vitamin K, also, is effective in lowering the blood pressure of hypertensive rats (*Ibid.* **2,** *240* (*1944*)).

T. R. Harrison, A. Grollman, and J. R. Williams (*Am. J. Physiol.* **128,** *716* (*1940*)) in 1940 reported the successful use of kidney extracts in treating hypertensive rats and in treating a few hypertensive patients (*J. Am. Med. Assn.* **115,** *1169* (*1940*)). Similar results were obtained in other laboratories. In the same year J. G. Peña and M. Villaverde (*Rev. cubana de cardiol.* **2,** *332* (*1940*))

used marine oil concentrates in the therapy of essential hypertension in man, and in 1942 G. E. Wakerlin, W. G. Moss, and E. L. Smith (*Science* **96,** *161* (*1942*)) applied similar concentrates in the treatment of the "Goldblatt dog." The favorable results were at first attributed to vitamin A but G. E. Wakerlin and W. G. Moss (*Proc. Soc. Exp. Biol. Med.* **53,** *149* (*1943*)) later showed that not all concentrates of vitamin A were effective. This observation has been confirmed and it is now generally accepted that the active principle in marine oils is not the vitamin. On the basis of the effects of saponification and oxidation, and of tests of various concentration procedures, A. Grollman (*J. Pharmacol. Exp. Therap.* **84,** *128* (*1945*)) has suggested that it is probably a derivative obtained by oxidation of a fatty acid or possibly of some other esterifiable substance. He has suggested, further, that it may be identical or of related chemical structure to the active substance in renal extracts.

H. Schwarz and W. M. Ziegler (*Proc. Soc. Exp. Biol. Med.* **55,** *160* (*1944*)) tested vitamin K preparations in rats rendered hypertensive by wrapping both kidneys with silk. They reported that 2-methyl-1,4-naphthoquinone (but not a water soluble derivative) significantly reduced the blood pressure of these animals. The vitamin preparations were given in daily doses of 5 to 10 mg. by intramuscular injection. On the basis of these results, the authors suggested trial of vitamin K in human hypertension. B. S. Oppenheimer, S. Soloway, and B. E. Lowenstein (*J. Mt. Sinai Hosp.* **11,** *23* (*1944*)) obtained similar results with 10 to 15 mg. daily doses of 2-methyl-1,4-naphthoquinone, using rats with cellophane wrappings applied to one or both kidneys. They reported this compound to be effective also in hypertensive dogs but in "relatively large" doses only.

W. G. Moss and G. E. Wakerlin (*J. Pharmacol. Exp. Therap.* **86,** *355* (*1946*)) have been unable to show any effect of vitamin K on hypertension in dogs. The animals which weighed 10 to 15 kg. were rendered hypertensive by the classic Goldblatt technic and were required to show a good to excellent grade of hypertension for a minimum period of five months prior to use as assay animals. Vitamin K was given orally to 4 animals and intramuscularly to one in daily doses of 60 mg. for six months. An additional animal received oral doses of 30 mg. daily for three months. None of these animals showed any significant changes in blood pressure. The authors concluded that vitamin K is without antihypertensive activity in dogs with renal hypertension. They had previously tested a vitamin B-complex concentrate, and vitamins C and E, as well as dried brewers' yeast and marine oil concentrates in similar hypertensive dogs. Only the marine oil concentrates gave positive results; of six lots of this material, excellent hypertensive activity was shown by one, slight activity by two, and no activity by three.

From the results of Moss and Wakerlin it appears that, while marine oil concentrates deserve further investigation, vitamin K is not a promising therapeutic agent for treatment of essential hypertension in man. Their dogs might have responded to larger doses, of course (60 mg. of the vitamin were given daily to each dog as compared with 5 to 15 mg. per rat per day in the reported successful tests on this animal) but it would seem that, at best, extremely high dosage would be required.

FAT EXCRETION IN MAN

The study of absorption and excretion of fat in man has been one of the most neglected of metabolic studies. Sporadic reports in the literature indicate that collecting and

examining feces of man is a task usually shunned. A report by E. E. Wollaeger, M. W. Comfort, J. F. Weir, and A. E. Osterberg (*Gastroenterology* **6,** *83 (1946)*) points a way to this study by establishing a reasonable foundation of control studies.

The purpose of the study was to compare the intake-excretion levels of fat in normal persons with those of patients who had undergone various types of gastric operations. Of the 13 subjects comprising the control group, 6 were in good health and the other 7 had duodenal ulcers which were not producing symptoms. Since results of study of these 13 subjects were similar they were used as a single control group. The diet consisted of bland foods of low bulk which contained 208 g. of fat, 97.5 g. of protein, and 140.5 g. of carbohydrate and furnished 2823 calories daily. All food was eaten each day and the menus did not vary from day to day. A vegetable gum of the tragacanth group was used as a bulk producer and was given in doses of 9 g. daily to prevent constipation. The studies were carried out for a period of three days.

Analytical procedures for determination of lipid content were carried out on wet feces rather than dried feces; a method essentially the same as that of Saxon was employed. The total amount of nitrogen was determined by means of the Kjeldahl procedure. Despite relatively great intakes of fat, little fat was lost in the stool. The largest amount of fecal fat lost by any member of the group was 13.6 g. per day, or 6.5 per cent of the fat ingested; the smallest amount of fat lost in the feces was 5.5 g., or 2.6 per cent of the fat ingested. The total amount of fecal solids varied from 15.5 to 40.4 g. a day and the average was 26.6 g. per day. The amount of nitrogen in the feces varied from 0.6 to 1.9 g. per day and the average was 1.2 g. daily.

In many previous studies of excretion of fat, the loss is expressed as the percentage of the weight of dried stool which is fat. When thus expressed the upper limit of normal when the subject is taking an average diet is considered usually to be 30 per cent. These studies indicate, however, that the amount of fat in the stools when the patient is on a diet which contains a large amount of fat may go well above 40 per cent without indicating abnormal loss of fat. On the other hand, in cases in which definite steatorrhea is present, such as may occur in sprue, it is not uncommon to find that fat constitutes less than 40 per cent of the weight of the dried stool. It appears, therefore, that measurement of the loss of fat in terms of percentage of the total fecal solids which is fat may be misleading unless information regarding dietary intake of fat is available.

A few authors have suggested that some patients who have undergone partial gastrectomy lose an excessive amount of fat in the stool. Others have suggested that abdominal symptoms, including diarrhea, rather marked loss of weight or inability to regain lost weight, may be associated with this excessive loss of fat. In a further study by E. E. Wollaeger, M. W. Comfort, J. F. Weir, and A. E. Osterberg (*Gastroenterology* **6,** *93 (1946)*) a group of patients who had undergone partial gastrectomy received the same diet that the control group received. This group was comprised of 10 men who had undergone partial gastrectomy for duodenal ulcer without complications. In all 10 subjects the entire cut end of the stomach was joined to the jejunum (Polya anastomosis). The period during which the studies were carried out was also three days. In comparison with the patients in the control group, these patients lost excessive amounts of fat in their stools. The difference of loss between the two groups was not great but was definite. The amount of fat lost, measured in grams per day, by those who had undergone gastrectomy ranged from 13.5 to 31.5 g. while the control subjects lost from 5.5 to 13.6 g. per day.

When the range of the percentage of ingested fat lost is compared the difference is also considerable. In the second group the range was from 6.5 to 15.1 per cent and in

the control group, from 2.7 to 6.5 per cent. The percentage of total fecal solids which was fat also was elevated in the group of patients who had undergone gastrectomy. The amount of fecal nitrogen fell within normal range and the amount of fecal solids was somewhat greater than it was in the control group.

In another group of 4 patients who had undergone partial gastrectomy severe distress followed ingestion of food and the patients could not gain weight. Members of this group with one exception were studied for three or four week periods and although they ingested a smaller amount of fat than the members of the control group, the percentage of ingested fat lost in the stools was invariably greater than it was in the control group. From these studies it is obvious that even when the diet contains a moderate amount of fat the deficiency present after partial gastrectomy allows excessive loss of fat. The authors present evidence to show that patients with the most severe symptoms following partial gastrectomy tend to lose a greater amount of fat in the stools.

In an effort to account for the failure of some patients to gain weight, a calculation of the caloric content of the feces was made. It was assumed that the nitrogen in the feces was in the form of protein and the carbohydrate content was disregarded. On this basis the range of loss among control subjects was from 70 to 170 calories per day, whereas among those who had undergone gastrectomy the range was from 122 to 673 calories per day. The importance of such loss of calories has not been studied further. Apparently unless these caloric losses are compensated for by increased caloric intake they may become significant in the problem of postoperative loss of weight.

Several factors might conceivably affect the production of an excessive loss of fat and nitrogen in the stools of persons who have undergone partial gastrectomy. The authors suggest as important factors the rapid emptying of the stomach, accelerated intestinal action, diminished flow of pancreatic secretion or bile, possible impairment of the mechanism which stimulates such flow, and incomplete mixture of food with digestive enzymes because of shunting of the gastric contents away from the duodenum.

These investigations not only show the importance of carefully establishing control values but point also to the wealth of knowledge of nutrition to be obtained by simple observations of normal persons and in abnormal clinical conditions.

INCORPORATION OF SULFIDE SULFUR INTO CYSTINE BY THE RAT

With the aid of radioactive and stable isotopes of sulfur, considerable information about the reactions of sulfur-containing amino acids was gained. It is now known that sulfur of methionine can be used for the formation of cystine *in vivo*, but that the beta- and gamma-carbon atoms of methionine are not utilized in the production of cystine from methionine. The carbon skeleton of the cystine formed can be derived from serine, one possible course of reaction being the combination of homocystine and serine to form the thio-ether cystathionine, which can undergo fission to yield cystine. However an alternative to this method of formation of cystine still appears likely to represent the true pathway of change undergone by the sulfur atom, since no proof has been adduced to show that homocystine is a naturally occurring metabolite.

The observation of C. V. Smythe and D. Halliday (*J. Biol. Chem.* **144,** *237 (1942)*) that the enzyme which catalyzes the breakdown of cysteine to pyruvic acid, ammonia, and hydrogen sulfide can also promote the incorporation of sulfide sulfur into cysteine, suggests a possible mechanism of formation of cystine *in vivo*. The enzyme which

catalyzes this reaction is known to be distinct from that which promotes the transfer of the sulfur of methionine or homocysteine to serine.

Following administration of sodium sulfide containing radioactive sulfur (S^{35}) all tissues of rats tested were found to contain some of the radioactive sulfur (*Nutrition Reviews* **4,** *206 (1946)*). The *in vitro* observation of Smythe and Halliday suggested the examination of cystine in the tissues of the rats which had received radioactive sulfur, for the presence of the labeled isotope. Such an examination is now reported by D. D. Dziewiatkowski (*J. Biol. Chem.* **164,** *165 (1946)*).

Radioactive sulfur was formed by the irradiation of carbon tetrachloride, and isolated as barium sulfate, which was reduced to barium sulfide and converted into sodium sulfide. The radioactive solution was diluted with ordinary sodium sulfide, and solutions prepared which contained approximately 1 mg. of sulfide sulfur per milliliter. A part of this was converted to barium sulfate and used for establishing the intensity of its radiation. Subsequent counts after intervals of a few days and weeks established that the half-life of the radioactive material was approximately 88 days, which is the value previously established for S^{35}.

Two young adult rats, one of each sex, were kept in metabolism cages and fed during a thirty minute period each day. Just after the daily feeding period on three consecutive days, each rat was given by stomach tube 0.15 ml. of pure bromobenzene, followed thirty minutes and six hours later by intraperitoneal injections of two 0.5 ml. portions of the solution of radioactive sodium sulfide. The injections of sodium sulfide were continued for eight days after the last dosing with bromobenzene, or for eleven days in all. The limitation on their daily period of feeding was discontinued after the fourth day of the experiment, and dog chow was then offered ad libitum. The rats were then killed and cystine was isolated from the hair, skin, muscle, and liver.

During the period of the experiment the daily urines of each rat were collected and pooled, and mercapturic acid (the conjugate of bromobenzene and cystine) was isolated from each specimen. The various specimens of mercapturic acid as well as cystine were tested with the radiation counter for their specific activity. The results showed that a small quantity of radioactive sulfur was incorporated into each sample tested, although even the smallest proportion of S^{35} found was shown to be significantly greater than could be caused by accidental contamination of the sample with radioactive sulfur. It appears then that the reaction observed by Smythe and Halliday *in vitro*, in which sulfide sulfur is built into the cysteine molecule, also occurs *in vivo* in the rat, although at only a slow rate.

The amount of sulfur thus used in the intact animal is so small that it is almost negligible in some cases. This was true even though dosing with bromobenzene caused depletion of the protein stores in general as shown by loss of weight, and of cystine in particular as shown by the synthesis of mercapturic acid. When bromobenzene treatment was discontinued, recovery in weight was observed and it was to be expected that fresh cystine would be formed during this period for incorporation into the newly formed protein. Nevertheless only a small amount of the S^{35} was present in the cystine isolated from body organs eight days after discontinuing the treatment with bromobenzene. It was concluded that the formation of cystine from sulfide sulfur accounts for only a very small proportion of the cystine in the proteins of the tissue examined.

VITAMINS IN SKIN DURING CARCINOGENESIS

The vitamin B content of tumor tissue has been discussed previously (*Nutrition Reviews* **1,** *271 (1943)*), but up to the present there has been little or no information on the vitamin content of tissues during carcinogenesis. E. L. Tatum, M. G. Ritchey, E. V. Cowdry, and L. F. Wicks (*J. Biol. Chem.* **163,** *675 (1946)*) have now reported on determinations of the biotin, choline, inositol, para-aminobenzoic acid, and pyridoxine content of mouse epidermis during methylcholanthrene carcinogenesis.

For making the assays, vitamin bioassay methods using mutant strains of Neurospora (G. W. Beadle and E. L. Tatum, *Proc. Nat. Acad. Sci.* **27,** *499 (1941)*) were developed or modified for use in determining the levels of these substances in the epidermis. Female Swiss mice 3 to 6 months old were used in the experiments, for which the mice were shaved over the entire back, using distilled water only to wet the hair. Groups containing 30 mice each received treatment for various lengths of time, with pure benzene in the control groups or with a 0.6 per cent solution of methylcholanthrene in pure benzene in the experimental animals. The solution and the solvent were painted on the skin with a fine camel hair brush, treatments being given three days a week until, in different groups, 1, 3, 6, 13, or 25 treatments in all had been administered.

When the treatments of any group of mice were completed, they were dry-shaved if necessary to remove any regrown hair, killed, and the epidermis of the treated area removed by blunt dissection as described by J. P. Baumberger, V. Suntzeff, and E. V. Cowdry (*J. Nat. Cancer Inst.* **2,** *413 (1942)*). All samples from the group were pooled and dried over phosphorus pentoxide at room temperature in the dark for sixty hours or more. A pooled sample contained from 0.4 to 0.8 g. of dried epidermis. For estimation of the vitamins the tissue was extracted and the vitamins liberated from combination by autoclaving with 4 Normal sulfuric acid at 15 pounds pressure for two hours, using 1 ml. of acid for 0.1 g. of dry material. The extracts were diluted, neutralized with bicarbonate to Congo red, brought to pH 5.5, then stored at 10°C. until assayed. During storage for one month no change occurred in the concentrations of the vitamins assayed. The vitamins were assayed by the growth of specific Neurospora mutants, which require the single addition of biotin, choline, inositol, para-aminobenzoic acid, or pyridoxine to the basic medium before growth of the respective mutants can occur.

No significant changes were observed in the levels of inositol, choline, or para-aminobenzoic acid. The pyridoxine content increased somewhat in mice treated either with methylcholanthrene solution or with benzene itself. The biotin content on the other hand decreased by about one-third after a single application of methylcholanthrene but not of benzene, and then maintained approximately the same low level during repeated paintings over sixty days. The reality of the biotin decrease is strengthened by the steady levels of the other factors maintained during the course of the experiment.

Several previous studies have been concerned with the vitamin content of tumor tissues (*Nutrition Reviews* **1,** *247, 271, 361 (1943)*), and some with specific findings of biotin changes (*Ibid.* **1,** *51, 280 (1943)*; **2,** *345 (1944)*). The exact significance of biotin to carcinogenesis or to tumor metabolism is not yet clear.

MICROBIOLOGIC ASSAYS OF VITAMINS AND AMINO ACIDS

Continued development of microbiologic assays for the estimation of vitamins and amino acids is still occurring at a rapid rate. Previous attention has been given to these methods (*Nutrition Reviews* **3,** *27* (*1945*); **4,** *113* (*1946*)) and references to earlier articles dealing with vitamins and amino acids have been cited.

J. L. Stokes, M. Gunness, I. M. Dwyer, and M. C. Caswell (*J. Biol. Chem.* **160,** *35* (*1945*); *Nutrition Reviews* **4,** *113* (*1946*)) developed a uniform assay for the ten essential amino acids, using one standard procedure, and measuring lactic acid production. Nine of the essential amino acids were assayed with Streptococcus faecalis, and phenylalanine with Lactobacillus delbrückii. Later, M. Gunness, I. M. Dwyer, and J. L. Stokes (*J. Biol. Chem.* **163,** *159* (*1946*)) showed that this basic method can be applied to the estimation of tyrosine.

Tyrosine assays are made with the organism L. delbrückii. Routinely the protein is hydrolyzed with alkali, 0.5 to 1.0 g. being treated with 10 ml. of 5 Normal sodium hydroxide and autoclaving in sealed ampules at 15 pounds pressure for ten hours as previously recommended for the assay of tryptophane. Only the naturally occurring l-tyrosine can be metabolized by L. delbrückii, the d-tyrosine being completely inactive. Hydrolysis of the sample with alkali causes complete racemization of the tyrosine present, so that the assay value must be multiplied by a factor of 2 to obtain the initial tyrosine content of the sample. Free tyrosine is less rapidly racemized than tyrosine combined in protein.

Results obtained by this method were reported for a number of proteins and also for a number of foods. The values for the foodstuffs compared well with earlier (chemical) figures in the literature, being in general slightly lower than those previously reported. As much as 50 per cent of the tyrosine in foods with a high carbohydrate content may be lost owing to humin formation if hydrolysis is carried out with acid, so that alkaline hydrolysis, which does not cause such a loss, is particularly necessary for food materials.

M. S. Dunn, M. N. Camien, S. Shankman, and H. Block (*J. Biol. Chem.* **163,** *577* (*1946*)) have shown also that L. fermenti 36 can be used as an assay organism for the estimation in protein hydrolysates of methionine, and for threonine (*Ibid.* **163,** *589* (*1946*)). Using a technic developed earlier in the same laboratory by M. S. Dunn, S. Shankman, and M. N. Camien (*Ibid.* **161,** *669* (*1945*)), these investigators compared the use of L. fermenti, L. arabinosus 17–5, and Leuconostoc mesenteroides, each with its own medium, for the assay of methionine. The most reliable results were obtained with L. fermenti 36 and incubating for two days at 35°. By this means casein appeared to contain 3.0 ± 0.1 per cent of methionine, and silk fibroin contained 0.14 ± 0.1 per cent, although it was recognized that before these can be regarded as true values, the extent of destruction of the methionine during the preliminary hydrolysis must be measured. The methionine value for casein was considered correct since it agreed closely with figures reported from ten laboratories employing seven different methods of analysis.

With an essentially similar medium and technic, the same organism was also used by these authors for the assay of threonine. For this amino acid, as well as for methionine, recovery tests were made by the addition of threonine to hydrolysates of casein, and assay was made of the threonine content of artificial mixtures of amino acids with very satisfactory results, the average deviation from 100 per cent recovery being ±3.5 per cent for the amino acid mixtures, ±5 per cent for casein hydrolysates, and ±9 per cent for fibroin hydrolysates. The

value found for the threonine content of casein was 4.3 per cent, and for silk fibroin 1.2 per cent. Consideration of earlier reports together with their own experience led the authors to conclude that the true values are probably 4.3 ± 0.2 and 1.2 ± 0.2 respectively, although here again these figures cannot be accepted finally until more is known about the extent of destruction of threonine during hydrolysis.

A report of an improved medium for microbiologic assays for the estimation of vitamins with Lactobacillus casei has been made by E. C. Roberts and E. E. Snell (*J. Biol. Chem.* **163,** *499 (1946)*). The chief quantitative component of the digest is enzymatically digested casein. Digestion is carried out by pancreatin for forty-eight hours at 37°, using a uniform suspension of 120 g. of Labco casein in 2 liters of 0.8 per cent sodium bicarbonate solution, and 600 mg. of pancreatin in about 20 ml. of distilled water. After adjusting to pH 6.0 by adding glacial acetic acid and filtering, the digest was stirred with 60 g. of activated charcoal to remove traces of various vitamins. To the final clear filtrate are added weighed amounts of sodium acetate, phosphate, glucose, cystine, uracil, adenine sulfate, guanine hydrochloride, para-aminobenzoic acid, biotin, calcium pantothenate, folic acid, niacin, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, and a simple salt mixture.

With this medium the general procedure is similar to that of the widely used method of E. E. Snell and F. M. Strong (*Ind. Eng. Chem. (Anal. Ed.)* **11,** *346 (1939)*). The chief advantage of the new medium is the rapid growth of the L. casei until maximum turbidity is reached in sixteen to eighteen hours, and the greater density of the bacterial suspension at the maximum. Thus in the assay of riboflavin the maximum dry weight of cells produced in the new medium was almost five times as great as in the Snell-Strong medium, and in the assay of folic acid it was about four times as great as in the medium used by L. J. Teply and C. A. Elvehjem (*J. Biol. Chem.* **157,** *303 (1945)*). When the incubation was continued for seventy-two hours, the acid production was also greater than with the Snell-Strong medium for assay of riboflavin, although the increase was much less than that in the turbidity.

The medium appeared to be satisfactory also for the estimation of niacin, pantothenic acid, biotin, and folic acid. For folic acid and riboflavin it was shown for a variety of food materials and tissues that almost identical results were obtained by the acidimetric titration and by the turbidity measurement, recoveries of the vitamins added to natural materials were satisfactory, and the results agreed closely with those obtained by older, slower methods. The new modification permits more rapid assays using turbidity measurements, and gives equally precise results as the older assays. It also permits the use of the single medium for the assay of a number of vitamins. It does not contain supplements of natural materials, although such supplements may in the future be of value for special purposes. L. arabinosus and L. pentosus also grow luxuriantly in the medium in the sixteen hour period, and presumably these organisms could be used for the rapid turbidimetric determination of niacin, pantothenic acid, and biotin.

QUANTITATIVE REQUIREMENTS OF THE CHICK FOR ISOLEUCINE, LEUCINE, AND VALINE

Various investigations on the essential and dispensable amino acids for the chick have been compared and discussed (*Nutrition Reviews* **3,** *100 (1945)*). Among the essential

amino acids, isoleucine, leucine, and valine were found to be essential for the maintenance of life, with the omission of any one resulting in an immediate loss in weight and eventual death.

C. R. Grau and D. W. Peterson (*J. Nutrition* **32,** *181 (1946)*) have studied the quantitative requirements of the chick for these amino acids. White Leghorn chicks were fed a commercial mash for the first ten days. From the tenth to the fourteenth day they were fed a conditioning diet containing casein 25 g., calcium gluconate 8 g., gelatin 7 g., cellulose (Cellu flour) 5 g., soybean oil 5 g., sardine oil as a source of vitamins A and D, natural mixed tocopherols, and the pure chemicals: choline chloride, inositol, cholic acid, 2-methyl-1,4-napthohydroquinone diacetate, thiamine, riboflavin, pyridoxine, niacin, calcium (d) pantothenate, biotin, folic acid, and finally glucose to make 100 g. When the chicks were 2 weeks old, groups of 4 closely matched chicks were selected. The basal experimental diet was identical with the conditioning diet except that the casein and gelatin were replaced with a mixture of commercially prepared amino acids. Sodium bicarbonate was added to neutralize the hydrochlorides of the basic amino acids. When the amount of the amino acid whose requirement was under investigation was reduced below that present in the mixture, an equal weight of glucose was added. The chicks were fed for ten days on the various diets containing altered amounts of isoleucine, leucine, and valine.

In the investigation of the isoleucine requirement, the best growth was obtained when the diet contained 1 per cent dl-isoleucine. Approximately 0.5 per cent of l(+)-isoleucine was found to give as good growth as 1 per cent of the dl-form, indicating that d(−)-isoleucine which is the unnatural form could not be used for the promotion of growth in the young chick.

As the leucine content of the diet was increased, growth increased until a maximum was reached when the diet contained 1.5 per cent l(−)-leucine. At all levels of leucine ingestion, the dl-form produced as good growth as the same amount of the l(−)-form. This would indicate that both the natural and unnatural forms of leucine can be utilized by the young chick.

In the study of the valine requirement, the best growth was observed when the diet contained 1.5 per cent of dl-valine. The ingestion of 0.7 per cent of l(+)-valine resulted in about as good growth as 1.5 per cent of dl-valine and a great deal more rapid growth than that observed in the chicks receiving 0.7 per cent of d(−)-valine, which is the naturally occurring form.

These studies mark a considerable advance in the science of nutrition in the eight years since the classic experiments of W. C. Rose (*Physiol. Rev.* **18,** *109 (1938)*) whose experiments indicated that rats will grow on diets containing amino acid mixtures as the sole source of nitrogen and that ten amino acids were required for the growth of the rat. At that time the chick could not be used for detailed studies on the amino acid requirement because of the numerous nutritional factors required by it which were not then available in purified or crystalline form. The rapid growth rate of the young chick and therefore the ease of depletion make it an ideal species for short term assays of the amino acid requirements. The determination of the requirements of the rest of the essential amino acids in the fashion described for isoleucine, leucine, and valine will produce a foundation from which the interrelation of the amino acids to each other and to other essential nutrients can be evaluated. The interrelation of tryptophane and niacin metabolism will be one of the most interesting to evaluate thoroughly because of the possible implications in human nutrition (*Nutrition Reviews* **3,** *267 (1945)*; **4,** *182 (1946)*).

FORMATION OF GLUCOSE FROM MESO-INOSITOL IN THE RAT

A number of attempts have been made to determine whether inositol is glucogenic in mammals, no doubt instigated by the isomerism of inositol with the hexoses. It had long been established that inositol injected in large doses may be excreted up to 50 per cent but the evidence did not indicate the fate of the unexcreted inositol. Attempts to show an increased excretion of glucose following administration of meso-inositol to diabetic or phlorhizinized animals gave contradictory results, but the search for newly formed glycogen in the livers of fasted animals given inositol was unsuccessful, and no increase in the respiratory quotient was found after inositol was given.

Unlike glucose, inositol was not observed to cause lactic acid formation when perfused through surviving livers, though an increase in lactic acid occurred simultaneously with the disappearance of inositol from muscle or liver mince, and lactic acid had been isolated from the urine of rabbits given inositol subcutaneously. From these conflicting observations, summarized by J. Needham (*Ergeb. Physiol.* **25,** *1 (1926)*) no conclusion could be drawn concerning the conversion of inositol to glucose.

Reopening of the question has however been caused by work on the structure of meso-inositol which has shown that cleavage of the inositol ring between a specific pair of carbon atoms would produce a molecule having the configuration of d-glucose (H. O. L. Fischer, *Harvey Lectures* **40,** *156 (1944-45)*). This stimulated M. R. Stetten and DeW. Stetten (*J. Biol. Chem.* **164,** *85 (1946)*) to attempt to demonstrate the glucogenic nature of inositol by using the more sensitive technics made possible by the employment of isotopes. As is pointed out by the authors, the quantity of glucose in the urine of a diabetic animal or of glycogen in the liver of a fasted animal will be significantly influenced only if relatively large amounts of the test substance are converted rapidly into glucose. If the test substance could be obtained in a stable form labeled with an isotope, its conversion to glucose in small amounts might be detected by the appearance of the isotope in the glucose excreted or the glycogen stored.

The previous experience of DeW. Stetten (*J. Biol. Chem.* **159,** *123 (1945)*) indicated that deuterio-glucose and deuterio-glycogen molecules contain carbon-bound hydrogen atoms which are stable in both chemical and biologic reactions. The study reported was therefore an attempt to introduce carbon-bound deuterium atoms into the molecule of meso-inositol, to feed such inositol, and to examine subsequently the glucose and glycogen of the animal for the presence of deuterium (isotopic hydrogen). The preparation of meso-inositol by the catalytic hydrogenation of hexahydroxy-benzene had been described previously, but attempts to repeat this synthesis failed. However, by treating inositol with platinum catalyst in deuterium oxide at 130 to 150° for periods of one to four weeks, deuterium was found to enter the carbon-bound positions of inositol to the extent of 4 to 17 per cent of the theoretical possibility. By means of this reaction, the authors obtained a sample of inositol which contained 1.75 atom per cent deuterium in the stable carbon-bound positions.

This isotopic inositol was tested by intraperitoneal injection into test animals (rats). Relatively large amounts were given, in view of the known urinary excretion of considerable fractions of the inositol. In a first test 2 g. of deuterio-inositol were injected in divided doses over a period of seven and a half hours into a fasted phlorhizinized rat and the urine was collected for twenty-four hours. The sample contained 2 g. of glucose, which was isolated as potassium gluconate, and contained 0.116 atom per cent deuterium. To test the possibility that this deuterium might be present in contaminating inositol from the urine, the gluconate was converted into the benzimidazole derivative, which would contain

0.116 × 11/16 = 0.080 atom per cent deuterium if all the deuterium was present in the gluconate and not in contaminants. The benzimidazole derivative was found to contain 0.075 atom per cent deuterium, confirming that the deuterium was nearly all present in the urinary glucose excreted by the rat. The source of this deuterium could only have been the injected deuterio-inositol.

From the concentration of the isotopic glucose obtained in this experiment, it was possible to estimate that a minimum of 7 per cent of the inositol administered had been converted into glucose. An attempt to demonstrate the same conversion in a rat rendered diabetic with alloxan was not successful because of the small quantity of glucose excreted after injection of the inositol. A third experiment, in which 6 rats which had been previously fasted were given inositol and killed three hours later, involved isolation of the muscle glycogen. This was found to be abundant, but contained so small a concentration of deuterium as to give a figure too low for satisfactory interpretation.

The experiment with the phlorhizinized rat does nevertheless appear to establish conclusively that meso-inositol can be converted to glucose, although it is not an efficient precursor. After the injection of 2 g. of inositol, only 140 mg. of isotopic glucose were recovered from the urine. Since such small amounts of glucose are produced from inositol, there is little wonder that previous methods not using the delicate technic of the isotopic "tag" failed to establish with certainty the production of any glucose from meso-inositol.

RIBOFLAVIN AND HUMAN NUTRITION

The amount of riboflavin required for normal human nutrition has not been determined with certainty although provisional allowances have been recommended by the Food and Nutrition Board of the National Research Council (*Nutrition Reviews* **3,** *287* (*1945*)). The problem of the recognition of specific ariboflavinosis in man (*Ibid.* **4,** *130* (*1946*)) and the interpretation of the relation between dietary intake and urinary excretion (*Ibid.* **3,** *235* (*1945*)) must be clarified before the requirement of the dietary essential can be established. The relation between intake and excretion has been complicated by the possibility that bacterial synthesis of riboflavin in the intestine may constitute a variable source of this nutrient (*Ibid.* **4,** *310* (*1946*)).

Recent studies indicate that fecal excretion is largely independent of the dietary intake and of the urinary excretion of riboflavin. In a continuation of an investigation of the excretion of B-complex vitamins, C. W. Denko *et al.* (*Arch. Biochem.* **11,** *109* (*1946*)) found that the fecal excretion of riboflavin was approximately 1 mg. on diets of natural foodstuffs containing extremes of from 1.84 to 0.32 mg. of this nutrient. The subjects were 7 young men who subsisted on a normal diet (1.84 mg. of riboflavin daily) for twelve weeks and then on a restricted diet (0.36 mg. of riboflavin daily) for five weeks. Five of the subjects continued on the restricted diet (0.32 mg. of riboflavin daily) for an additional ten weeks. During the latter period, the other 2 subjects received a supplemented diet (1.98 mg. of riboflavin daily). The urinary excretion of the vitamin dropped sharply with the ingestion of the low-riboflavin food mixture and increased in the last period in the 2 subjects supplied the vitamin supplement. The fecal excretion exceeded the urinary excretion in all periods and was three times greater than the intake during the low-riboflavin period.

In contrast to this demonstration of the absence of an effect of dietary riboflavin on fecal excretion is the positive effect of the character of the foodstuffs in the diet (M. L. Hathaway and D. E. Lobb, *J. Nutrition* **32,** *9* (*1946*)). These investigators compared the fecal and urinary excretion in 3 adult women who ate a synthetic diet (1.09 mg. of riboflavin daily) for forty-nine days and, after a thirty-day

interval on a mixed natural diet, consumed an experimental mixture of natural foodstuffs (1.33 mg. of riboflavin daily) for twenty days. The average urinary excretion was approximately 15 per cent of the intake in both periods but the fecal eliminations on the synthetic and natural diets were 28 and 84 per cent, respectively, of the intakes. In other words, the eating of natural foodstuffs was associated with the excretion of three and one-half times as much fecal riboflavin as the consumption of a synthetic mixture having a similar riboflavin content. Sixty to 70 per cent of the fecal riboflavin consisted of the free form. The basal synthetic mixture was the same as that used by V. A. Najjar and co-workers (*J. Am. Med. Assn.* **126,** *357 (1944)*) and had the following composition: casein, 72; Crisco, 128; Dextri-maltose, 200; and salt mixture, 20. It is reasonable to believe that the greater fecal excretion of riboflavin on the natural diet was the result of increased synthesis by intestinal bacteria because the total output in 2 of the 3 subjects exceeded the intake, and in one subject the fecal excretion alone nearly equaled the intake.

The utilization of riboflavin has also been studied by M. V. Davis, H. G. Oldham, and L. J. Roberts (*J. Nutrition* **32,** *143 (1946)*) who have reported extensive observations on the relation of intake to urinary and fecal elimination in 12 normal adult women during experimental periods lasting from three to eight months. The results are illustrated by the following representative data on one subject who continued on the experiment for the entire eight months. After a preliminary seven-day period of self-selected foodstuffs (pork and liver excluded from the dietary), a diet supplying 0.54 mg. of riboflavin daily (0.25 mg. per 1000 calories) was eaten for one hundred and six days (Period 1). At this time the riboflavin intake was increased to 1.01 mg. daily (0.47 mg. per 1000 calories) for forty-five days (Period 2) and then to 1.24 mg. daily (0.65 mg. per 1000 calories) for forty-one days (Period 3). These levels were obtained by selection of ordinary foodstuffs. During the next fourteen days (Period 4), riboflavin (6.0 mg.) was administered as a supplement and the total daily intake was 7.22 mg. A final twenty day period on an intake of 1.26 mg. (0.68 mg. per 1000 calories) completed the experiment (Period 5). Throughout the investigation, seventy-two hour collections of urine and feces were made each week except for the first week after each dietary change. The excretion of riboflavin in one hour fasting urines and the "per cent return" in four hour urines after the administration of a test dose (0.02 mg. per kilogram of body weight) were determined in the last week of each period. Supplements of calcium, iron, vitamin A, and ascorbic acid were given throughout the study. The diets supplied 49 to 62 g. of protein, 9.7 to 14.3 mg. of niacin, and 2.3 to 4.7 mg. of pantothenic acid daily. Thiamine was restricted because this vitamin as well as riboflavin was under investigation. The thiamine content of the diet increased from 0.14 in the first period to 0.54 mg. per 1000 calories in the final period. During the two weeks of riboflavin supplementation (Period 4), 4 mg. of thiamine, 10 mg. of niacin, and 0.5 mg. of pantothenic acid were supplied daily.

The urinary excretion of riboflavin leveled off within ten days after each dietary change. The average values for Periods 1, 2, and 3 (riboflavin levels of 0.54, 1.01, and 1.24 mg. daily) were 0.08, 0.14, and 0.23 mg. The output increased with the intake and represented 14 to 19 per cent of the daily supply. During the first and second weeks of supplementation (Period 4), 55 and 69 per cent, respectively, of the intake (7.22 mg. daily) appeared in the urine. Any excess resulting from this relatively large supply quickly disappeared because the urinary excretion during Period 5 was only slightly higher than that of Period 3. The fecal output was not measured in Period 4 but in all other periods it ranged from 0.5 to 0.65 mg. daily and appeared independent of the dietary level. It regularly exceeded

the urinary excretion and nearly equaled the intake in Period 1.

The elimination of riboflavin in one hour fasting urines in Periods 1, 2, and 3 varied with the dietary level and amounted to 0.008, 0.012, and 0.015 mg. It is significant that these values were less than that of the preliminary period (0.017 mg.) in which the food was largely unrestricted. As in the case of the twenty-four hour excretion, the fasting output in Period 5 (0.014 mg.) was only slightly higher than that of Period 3, indicating that there was no holdover effect of the intervening period of high supplementation. These levels in fasting (twelve hour) urines were somewhat higher than those reported by Hathaway and Lobb for similar riboflavin intakes. Both groups found that the fasting rate of excretion was more constant per unit of time than per unit of urine volume which is in disagreement with the conclusion of V. H. Feder, G. T. Lewis, and H. S. Alden (*J. Nutrition* **27,** *347 (1944)*). The test dose response followed the same pattern as the fasting excretion. It dropped sharply from 20 per cent in the preliminary period to 4 per cent in the low-riboflavin period (Period 1) and then increased with the riboflavin intake to 13 per cent in Period 3 and 10 per cent in Period 5.

No clear evidence of ariboflavinosis was observed in the subjects who remained on the low riboflavin diet (0.27 to 0.29 mg. per 1000 calories) for over one hundred days. Davis *et al.* concluded from this study that the daily intake of approximately 1 mg. of riboflavin (0.5 mg. per 1000 calories) satisfies the needs of normal young women. This conclusion was based on the fact that 40 per cent of the additional riboflavin supplied in Period 3 was excreted in the urine, whereas only 11 per cent of the increment in Period 2 was eliminated. Furthermore, relatively small increases in the fasting excretion and in the test dose response occurred in Period 3 coincident with the increase in riboflavin intake from 1.01 to 1.24 mg. daily. A minimum requirement of 0.5 mg. of riboflavin per 1000 calories for adult women is in accord with the observations of R. D. Williams, H. L Mason, P. L. Cusick, and R. M. Wilder (*J. Nutrition* **25,** *361 (1943)*) and is considerably less than the recommendations of W. H. Sebrell, R. E. Butler, J. G. Wooley, and H. Isbell (*Pub. Health Reports* **56,** *510 (1941)*).

These investigations of the utilization and excretion of riboflavin in human subjects emphasize the direct influence of the dietary supply on urinary elimination and the absence of a relationship between intake and the fecal excretion. The latter varies with the type of food mixture, presumably being greater if fecal contents favor the growth of microorganisms that synthesize the vitamin. Whether any riboflavin of intestinal origin is absorbed and utilized is not certain from these experimental data. The fact that the fecal output may exceed the intake on low-riboflavin diets indicates its partial, if not entire, nondietary origin and argues against inclusion of data on fecal elimination in estimating balance between intake and output. The urinary excretion normally ranges from 10 per cent on low to 70 per cent or more on high intakes of riboflavin. If none or little of the dietary supply escapes absorption in the intestine, an appreciable amount remains unaccounted for. If most of the absorbed vitamin which is not later found in the urine is used to replace the tissue supply, it would appear that tissue metabolism results in the destruction of relatively large amounts daily. It is possible that the concentration of riboflavin in the body increases if the dietary supply is abundant. Bacterial destruction of some dietary riboflavin in the intestine is also a possibility. The extent of the riboflavin "wear and tear" metabolism, the capacity of the body to store riboflavin, and the exact influence of intestinal bacteria in depleting or augmenting the dietary supply are questions which must be answered before the real significance of the urinary elimination can be understood.

NOTES

Letter to the Editor

Dear Sir:

I have read the review of my paper with the late Prof. I. J. Kligler and H. Herrnheiser ("Biotin Deficiency and Susceptibility to Infection with Salmonella") in the August, 1946 issue of Nutrition Reviews. I feel that some statements made by the reviewer are apt to throw a false light on the conclusions of our paper.

(1) The reviewer considers that the higher susceptibility of the biotin deficient rats may be due to a "possible occurrence of multiple vitamin B-complex deficiencies on a restricted rice-yeast diet" and he remarks "that actual quantities of rations consumed were not indicated." In our paper, however, we wrote: "Since diminished caloric intake in the biotin deficient group became apparent only in the sixth week of deficiency feeding, the enhanced susceptibility of the biotin deficient rats cannot be attributed to diminished food intake." Actually, the susceptibility tests were performed after two to four weeks on diet, before the food consumption and, therefore, the B-vitamin intake declined.

(2) In these tests 120 deficient and 120 control rats were tested. One hundred and thirteen deficient and 61 control rats were found to be infected. One hundred deficient rats out of 120 (and not out of 113, as the reviewer writes), and 54 control rats out of 120 (and not out of 61) showed positive mesenteric glands cultures.

(3) In our observations on spontaneous Salmonella epidemics in mouse populations 176 mice kept on the deficient diet and 176 controls were exposed to the infection. Forty-one mice of the first group and 16 control mice died during the observation period. The organs of 48 were cultured. In every case Salmonella was found. Among the surviving mice 120 out of 135 = 89 per cent (and not 114 out of 120 = 95 per cent, as the reviewer states) deficient mice and 52 out of 160 = 33 per cent (and not 45 out of 52 = 86.5 per cent) control mice were found to be infected at the end of the observation period.

I think, therefore, that our conclusion that biotin deficiency diminishes in rats and in mice the resistance to Salmonella infection, is justified.

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Antipellagra Activity of N′-Methylnicotinamide

Identification of N′-methylnicotinamide with the fluorescent F_2 excretion product of niacin has made it important to determine whether this compound possesses antipellagra activity. L. J. Teply, W. A. Krehl, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **58,** *169* (*1945*)) could not demonstrate any appreciable activity of this compound for blacktongue in dogs. On the other hand, V. A. Najjar *et al.* (*Bull. Johns Hopkins Hosp.* **74,** *406* (*1944*)) appeared to demonstrate antiblacktongue activity for N′-methylnicotinamide, and G. A. Vance (*Ibid.* **77,** *393* (*1945*)) described the amelioration of dermatitis and glossitis in a case of pellagra upon treatment with this compound. Unfortunately, this single case appeared to be one of mixed deficiencies, and certain psychomotor disturbances actually appeared to become more severe upon administration of N′-methylnicotinamide. Further difficulty was involved in interpretation of the therapy by preliminary treatment of the patient with 20 mg. of nicotinamide intravenously.

V. A. Najjar and C. C. Deal (*J. Biol. Chem.* **162,** *741* (*1946*)) chose to resolve this problem by application of a dual set of criteria. They anticipated that if N′-methylnicotinamide were split by the rat, then excessive fat deposition in the liver might be

prevented or decreased, on an appropriate diet, by the methyl groups freed. Furthermore, the liberated nicotinamide should result in its increased excretion. Experimentally, control rats were fed the Griffith and Wade diet known to produce fatty livers, while others were fed this diet with the addition of 1 or 2 per cent of N′-methylnicotinamide.

The excretion of nicotinamide was observed in an experiment in which the diet contained 1 per cent N′-methylnicotinamide over a period of three months. During this period the average daily excretion of nicotinamide in 4 rats was 11.8 micrograms, while in the absence of N′-methylnicotinamide 4.5 micrograms were excreted. The effect of this compound fed at a 2 per cent level on the liver fat of rats was also observed. The liver fat contents of 4 rats fed this compound were 24.5, 8.2, 10.6, and 23.5 per cent, as compared with 36.1, 39.1, 39.7, and 34.7 per cent in the control animals.

These results appear to prove the objective that N′-methylnicotinamide is demethylated in the rat, and the investigators promise further confirmation of these experiments with a larger number of animals. Yet there may be deficiencies in this experimental approach which will require further investigation to provide incontestable proof of the availability of N′-methylnicotinamide as a source of nicotinamide. For example, in the nicotinamide excretion experiments, if only 5 g. of ration were ingested daily, a nicotinamide impurity of less than 0.02 per cent in the N′-methylnicotinamide might increase the daily excretion of nicotinamide by the 7.3 micrograms noted. Rigid proof of the purity of the N′-methylnicotinamide is therefore demanded to make the excretion data completely valid. Regarding the data on liver fat, there was considerable variability in the experimental group, 2 of the rats possessing a markedly lower liver fat content. This suggests the possibility that the food intake of these rats was low due to the unpleasant bitter flavor imparted to the diet by N′-methylnicotinamide, and it is well known that a decrease in the food intake will delay or lessen the severity of fatty liver. Here again, for rigid proof of the availability of N′-methylnicotinamide as a source of methyl groups, a technic involving paired feeding control of dietary intake, or the use of "tagged" methyl groups would be desirable.

Satisfactory proof for the demethylation of N′-methylnicotinamide and its antipellagra activity is therefore still incomplete.

The Conversion of Carotene to Vitamin A

It has been suggested that the biologic conversion of beta-carotene to vitamin A involves oxidation at the central double bond of the molecule with subsequent reduction of the vitamin A aldehyde. Despite occasional claims to the contrary, it has not been possible to produce this conversion by enzymes or tissue extracts, nor until recently by chemical means. R. F. Hunter and N. E. Williams (*J. Chem. Soc. (London) p. 554 (1945)*) have apparently succeeded in the chemical conversion by using hydrogen peroxide as the oxidizing agent and aluminum isopropoxide for reduction of the vitamin A aldehyde. The resulting material was purified by chromatographic methods to give a very small yield of vitamin A, identified by spectrographic and biologic tests.

ERRATA

Vol. 4, No. 12, page 367. Insert page *91* after Biotin, role, in metabolism of pantothenic acid

Vol. 4, No. 12, page 364. Add *357* after Stetten, DeW., Jr.

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